ANTIFUNGAL ACTIVITY OF SL-1, A β-NITROSTYRENE TYPE PIGMENT AND ITS SYNTHETIC CONGENERS

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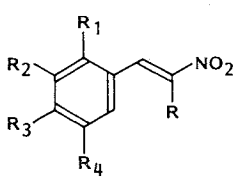
SL-1 (**1a**, Fig. 1) is a reddish brown pigment present in a saframycin-nonproducing mutant of *Streptomyces lavendulae* No. 314.[1,2] In our studies on the antimicrobial activity of the SL-1 pigment, we observed characteristic activity against dermatophytes such as *Trichophyton mentagrophytes*.[1] In this paper, we report antifungal activity of thirteen newly synthesized congeners of SL-1 (Fig. 2).

A few dihydroxybenzene SL-1 congeners (**1b**~**1d**) were obtained as followed. Protected benzaldehyde (**3a**)[1] was condensed with nitroethane to afford β-nitrostyrene[3,4] which was hydrolyzed with concentrated hydrochloric acid to yield the catecol (**1b**: MP 145~147°C; UV λ_{max} nm (log ε) 234 (3.84), 260 (3.85), 366 (4.09); IR ν_{max} (KBr) cm^{-1} 3510, 1635, 1605, 1590, 1490, 1400; MS *m/z* 195 (M^+); elemental analysis, calcd for $C_9H_9NO_4$: C 55.38, H 4.65, N 7.18, found: C 55.21, H 4.62, N 6.90; ^1H NMR (CD_3OD) δ 2.38 (3H, s), 6.75 (1H, s), 6.82 (1H, br s), 7.85 (1H, br s)) in 71.3% overall yield. Compound **1c** (MP 176~178°C; UV λ_{max} nm (log ε) 240 (sh, 3.66), 268 (3.86), 308 (4.01), 396 (3.79); IR ν_{max} (KBr) cm^{-1} 3320, 1620, 1590, 1500, 1450; MS *m/z* 181 (M^+); elemental analysis, calcd for $C_8H_7NO_4$: C 53.04, H 3.90, N 7.73, found: C 53.31, H 4.01, N 7.12; ^1H NMR (CD_3OD) δ 6.75 (3H, m), 7.80 (1H, d, J=14 Hz), 8.10 (1H, d, J=14 Hz)) was prepared from 2,5-dihydroxybenzaldehyde (**3b**) in 3 steps using the previous synthetic route of SL-1 (**1a**) in 60.8% overall yield. Compound **1d** was a gift from Dr. R. ROYER (Service de Chimie de l'Institut Curie, CNRS, France).

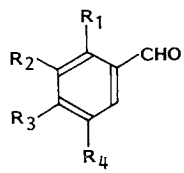
Various di- and tri-methoxyarene SL-1 congeners (**2a**~**2h**) were prepared from the corresponding benzaldehydes in the conventional manner.[3~5] MIC was determined by the agar dilution method using Bacto-Sabouraud dextrose agar (Difco) for fungi and Sensitivity disk agar (Eiken) for bacteria. *In vitro* activity of compounds **2a**~**2i** was determined from the size of the inhibition zone, using paper disks and *T. mentagrophytes* as test organism.

SL-1 (**1a**) showed moderate activity against Gram-positive and Gram-negative bacteria (Table 1). **1a** was also active against fungi with relatively low MIC values against *T. mentagrophytes* and *Trichophyton violaceum*. The activity of com-

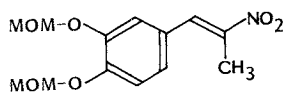
Fig. 1. Dihydroxybenzene SL-1 congeners.



1a R=R1=R4=H R2=R3=OH
1b R=CH3 R1=R4=H R2=R3=OH
1c R=R2=R3=H R1=R4=OH
1d R=R1=R3=H R2=R4=OH



3a R1=R4=H R2=R3=O-MOM
3b R1=R4=OH R2=R3=H
3c R1=R2=OCH3 R3=R4=H



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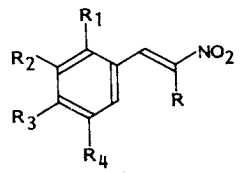
MOM: Methoxymethyl.

pound **1c** was slightly lower than that of **1a** against fungi and **1d** was almost inactive. These data indicate that the 3,4-dihydroxy group is essential for the exhibition of antimicrobial activity. Interestingly, the activity was strengthened by the introduction of a methyl group in the side chain as shown in compound **1b**, and actually all strains of dermatophytes were inhibited at the concentration of 3.13 μg/ml. Since these dihydroxy β-nitrostyrene compounds (**1a**~**1d**) were relatively unstable in solution, we turned our attention to the preparation of various di- and tri-methoxyarenes (**2a**~**2h**). Their antifungal activity is shown in Table 2. Among the eight compounds tested, 1,2-dimethoxy-4-(2-nitroethenyl)benzene (**2e**) was most active, followed by compound **2f**, on the basis of antifungal activity (zone diameter) against *T. mentagrophytes*. Substitution of 1, 2 position with methoxy or methyl group seems essential for the potentiation of antifungal activity in this class of compounds. Since our results (Table 1) showed that methyl group introduced in the side chain exhibits slightly higher antimicrobial activity, we also prepared compound **2i** (MP 75~77°C; IR ν_{max} (KBr) cm^{-1} 1670, 1590, 1525, 1485, 1435; MS *m/z* 223 (M^+); elemental analysis, calcd for $C_{11}H_{13}NO_4$: C 59.18, H 5.87, N 6.28, found: C 59.06, H 5.94, N 6.22; ^1H NMR ($CDCl_3$) δ 2.38 (3H, d, J=0.9 Hz), 3.85 (3H, s), 3.90 (3H, s), 6.91 (1H, dd, J=7.9 and 1.5 Hz), 6.99 (1H, d, J=7.9 and 1.5 Hz), 7.12 (1H, t, J=7.9 Hz), 8.23 (1H, br s)) by condensation of 2,3-dimethoxybenzaldehyde (**3c**) with nitroethane in the presence of sodium acetate at reflux for 2 hours; yield was

Fig. 2. Various di- and tri-methoxyarenes (**2a**~**2i**).



Compounds	R	R_1	R_2	R_3	R_4	MP (°C)
2a	H	OCH_3	H	OCH_3	H	108~108.5
2b	H	H	OCH_3	H	OCH_3	132.5~134
2c	H	OCH_3	CH_3	OCH_3	OCH_3	121~123
2d	H	OCH_3	H	H	OCH_3	121~123
2e	H	OCH_3	OCH_3	H	H	81~82
2f	H	OCH_3	H	OCH_3	OCH_3	132~133
2g	H	OCH_3	CH_3	OCH_3	H	103~104
2h	H	H	OCH_3	OCH_3	H	140~141
2i	CH_3	OCH_3	OCH_3	H	H	75~77

Table 1. Antimicrobial activity of SL-1 and its congeners.

Microorganisms	SL-1 and its congeners (MIC μg/ml)			
	SL-1 (**1a**)	**1b**	**1c**	**1d**
Corynebacterium xerosis IFM 2057	12.5	6.25	25.0	100.0
Escherichia coli NIHJ JC2	25.0	25.0	12.5	>100.0
Micrococcus luteus IFM 2066	12.5	6.25	50.0	50.0
Mycobacterium sp. 607 IFM 2051	50.0	12.5	50.0	>100.0
Staphylococcus citreus IFM 2025	12.5	6.25	50.0	100.0
S. aureus 209P IFM 2014	12.5	3.13	6.25	100.0
Candida albicans 1001	100.0	100.0	>100.0	>100.0
C. parapsilosis IFM 40020	100.0	50.0	>100.0	>100.0
C. stellatoidea IFM 40086 (*C. albicans*)	100.0	50.0	>100.0	>100.0
C. tropicalis IFM 40018	100.0	50.0	>100.0	>100.0
Cryptococcus neoformans IFM 40038	100.0	25.0	>100.0	>100.0
Aspergillus niger IFM 40606	>100.0	>100.0	>100.0	>100.0
Epidermophyton floccosum IFM 40770	25.0	3.13	25.0	>100.0
Paecilomyces variotii IFO 30539	100.0	25.0	>100.0	>100.0
Sporothrix schenckii IFM 40751	25.0	12.5	12.5	25.0
Trichophyton mentagrophytes 13	12.5	3.13	25.0	>100.0
T. mentagrophytes 14	6.25	3.13	6.25	100.0
T. mentagrophytes 15	25.0	3.13	25.0	>100.0
T. mentagrophytes 16	25.0	3.13	25.0	>100.0
T. rubrum IFM 40768	12.5	3.13	25.0	>100.0
T. violaceum IFM 40738	6.25	3.13	50.0	>100.0

MIC was determined by the agar dilution method using Sensitivity disk agar (for bacteria) and Sabouraud dextrose agar (for fungi).

Table 2. Structure-activity relationship of various di- and tri-methoxyarenes.

Compound	Inhibition diameter (mm)
2a	27
2b	23
2c	27
2d	23
2e	48
2f	23
2g	30
2h	27

Agar plates seeded with spores of *Trichophyton mentagrophytes* 14 were used.

Inhibition diameter around the paper disk containing 50 μg of each compound was determined 72 hours after incubation at 27°C (mean diameter of 4 plates).

Table 3. Comparison of antifungal activity of synthesized active β-nitrostyrenes **2e** and **2i**.

Microorganisms	MIC values (μg/ml)	
	2e	**2i**
Epidermophyton floccosum IFM 40770	0.2	0.39
Microsporum gypseum IFM 40766	1.56	1.56
Trichophyton mentagrophytes 24	1.56	1.56
T. mentagrophytes 14	1.56	1.56
T. violaceum IFM 40738	1.56	1.56
T. rubrum IFM 40768	0.78	0.78

MIC was determined by the agar dilution method using Sabouraud dextrose agar.

88%. Comparison of the MIC values of **2e** and **2i** shown in Table 3 reveals almost the same antifungal activity, and all strains of dermatophytes were inhibited at the concentration of 0.2 to 1.56 μg/ml. These data indicate that a methyl group introduced in the side chain of **2e** has no effect on the potentiation of antifungal activity, although it does have in dihydroxybenzene SL-1 congeners. Throughout the present experiments, we were able to obtain **2e** which has 5 to 20 times higher antifungal activity against dermatophytes than that of parent compound, SL-1. Further studies on the preparation of new derivatives of **2e** having even more potent antifungal activity are now in progress in our laboratory and the results will be reported elsewhere.

References

1) MIKAMI, Y.; K. TAKAHASHI, K. FUKUSHIMA, K. YAZAWA, T. ARAI, A. KUBO, N. SAITO & N. KAWAKAMI: A new pigment from *Streptomyces lavendulae*. J. Antibiotics 40: 385~387, 1987

2) MIKAMI, Y.; K. TAKAHASHI, K. YAZAWA, T. ARAI, M. NAMIKOSHI, S. IWASAKI & S. OKUDA: Biosynthetic studies on saframycin A, a quinone antitumor antibiotic produced by *Streptomyces lavendulae*. J. Biol. Chem. 260: 344~348, 1985

3) BUCHI, G. & C. MAKI: Nitro olefination of indoles and some substituted benzenes with 1-dimethylamino-2-nitroethylene. J. Org. Chem. 42: 1784~1786, 1977

4) SINHABABU, A. K. & R. T. BORCHARDT: Silica gel-assisted reduction of nitrostyrenes to 2-aryl-1-nitroalkanes with sodium borohydride. Tetrahedron Lett. 24: 227~230, 1983

5) RAIFORD, L. C. & D. E. FOX: Condensation of vanillin substitution products with nitromethane. J. Org. Chem. 9: 170~177, 1944